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Genetic Fine Mapping of the Gene for Nonsyndromic Congenital Retinal Nonattachment

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Autosomal recessive nonsyndromic congenital retinal nonattachment (NCRNA) comprises congenital insensitivity to light, massive retrolental mass, shallow anterior chamber, microphthalmia, and nystagmus in otherwise normal individuals. Polymerase chain reaction-based linkage analyses of polymorphic microsatellite markers in the 10q21 region on DNA samples from 106 individuals provide evidence that the NCRNA locus is within an interval of ~0.6–1.5 cM, flanked by the markers D10S522 and D10S1418. Haplotype analysis demonstrated a unique founder haplotype shared by 100% of the NCRNA chromosomes. These results indicate a founder effect and the strong possibility of a single mutation as the cause of the disease in the affected population. Based on these findings, it is now possible to provide relatively accurate carrier detection and prenatal diagnostic testing for families with NCRNA based on close flanking markers and the capacity to identify NCRNA chromosomes by their haplotypes. Am. J. Med. Genet. 92:220–223, 2000.
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INTRODUCTION

Nonsyndromic congenital retinal nonattachment (NCRNA) is a heterogeneous group of retinal disorders, autosomal dominant and recessive types having been reported [Phillips, 1973; Warburg, 1979; Weve, 1938]. One autosomal recessive form of NCRNA (MIM 221900), is a severe form of retinal defect characterized by congenital insensitivity to light, massive retrolental mass, shallow anterior chamber, microphthalmia and nystagmus in otherwise normal individuals [Ghiasvand et al., 1998]. The ancestors of the Iranian Kurds that constitute our study group settled on the plains of northeastern Iran among the Persians a few centuries ago; until recently their descendants remained genetically isolated because of linguistic isolation. While relatively rare in other populations, the prevalence of NCRNA in this group was estimated to be 1.1% with a carrier frequency estimate of about 20% [Ghiasvand et al., 1998]. For this reason fine structure mapping of the disease gene and development of a predictive test for the prevention of the disease in the affected population is of paramount importance.

Using unique short tandem repeat polymorphic markers (STRPs), pooling strategy [Sheffield et al., 1995], homozygosity mapping [Lander and Bostein, 1987], and DNA samples from 36 individuals from the study group, we previously assigned the NCRNA locus to chromosome band 10q21 [Ghiasvand et al., 2000]. Transmission disequilibrium test *P*-values for 10q21 markers D10S1225 and D10S1418 in this study were 0.0000021 and 0.000021, respectively.

To utilize the detectable meiotic recombination events in the affected population for determining the NCRNA interval and to examine the evident founder effect in this population, linkage analyses with 12 additional STRPs in the 10q21 region, and DNA samples from 70 additional individuals from the same affected population, were used. The 12 additional STRPs (D10S1211, D10S1241, D10S1422, D10S502, D10S1219, D10S1646, D10S1670, D10S522, AFM074XE1, AFMa130ZC5, D10S1418, D10S676) from the chromosome band 10q21 were used to construct the disease

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haplotype. In this study both the affected and the unaffected sibs and their parents were studied for possible historic and for new recombination events in the NCRNA region. Our data provide evidence that NCRNA locus is in a 10q21 segment of ~0.6–1.5 cM, flanked by the markers D10S522 and D10S1418.

FAMILIES AND METHODS

Families

Twenty NCRNA families from a founding Iranian population including 25 affected individuals, 46 of their normal sibs, and 35 of their normal parents were studied. The pedigree transmitting autosomal recessive NCRNA has been described elsewhere [Ghiasvand et al., 1998]. Informed consent was obtained from all participants of the study or their guardians. The research was conducted under the Institutional Review Board (IRB) approved guidelines.

DNA Analysis

Genomic DNA was prepared from blood samples using a standard "salting out" protocol [Miller et al., 1988] and quantitated spectrophotometrically. A set of 12 microsatellite polymorphic markers in the 10q21 region was typed with the DNA samples from 106 members of the Iranian population. The markers were di-, tri- or tetranucleotide repeat polymorphisms and primer sequences were obtained from the Genome database. The primers were synthesized in our laboratory using DNA Synthesizer Model 392/394 (Applied Biosystems, Foster City, CA).

PCR reactions using 32P labeled primers were run under the following conditions: approximately 10 ng of DNA; 1.25 μL PCR buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3); 300 μM each of dCTP, dGTP, dTTP, and dATP; approximately 2.5 pmol each primer; 0.25 U Taq polymerase (Boehringer) in a total volume of 4 μL. PCR reactions with one primer pair were run for ~30 cycles of 94° for 30 s, 55° for 40 s, and 72° for 40 s after an initial 3 min at 94°.

PCR products were separated by electrophoresis on 6% denaturing polyacrylamide gels containing 7.7 M urea. The allelic polymorphism was visualized by overnight autoradiography using Kodak XAR film. The developed gels were scored, and the results were stored using DataEntry, a Macintosh Hypercard genotype database application (from Six Ponds Software). This software was also used to generate the haplotypes for all the individuals studied. All genotype errors were checked by retyping.

RESULTS

Fine Localization of NCRNA Gene and a Unique Founding Haplotype

To find the closest flanking markers of the disease gene-containing region and to construct the disease haplotype, we analyzed the genotypes of 106 individuals from the study population for the 12 markers in the NCRNA region. The haplotypes of all the individuals were analyzed to (1) locate possible historic and new crossovers between the NCRNA locus and any of the markers in the disease haplotype and (2) estimate the number of independent NCRNA mutations in the study population, based on the number of different haplotypes transmitted with the disease.

A unique founder disease haplotype with a conserved set of 12 marker alleles, covering ~9 cM on 10q21, was observed. Due to a number of recombination events the disease haplotype has become shorter on both sides (Fig. 1). Analysis of the disease haplotype segregating in the affected population revealed that in addition to the historic 12-marker-long founder disease haplotype, called haplotype a, there are also 7 types of recombinant disease haplotypes associated with the disease locus (Fig. 1):

(1) Haplotype b is recombinant for marker D10S676, indicating that NCRNA lies proximal to D10S676.
(2) Haplotype c is recombinant for marker D10S676 on the distal side, and is also recombinant for D10S502 and other proximal markers, indicating that NCRNA lies distal to D10S502.
(3) Haplotype d is recombinant for marker D10S676 on the distal side and is also recombinant for D10S1219 and other proximal markers, indicating that NCRNA lies proximal to D10S676 and distal to D10S1219.
(4) Haplotype e is also recombinant for marker D10S1219 and other proximal markers confirming that NCRNA lies distal to D10S1219.
(5) Haplotype f is recombinant for STRPs D10S676 and D10S1418, indicating that NCRNA lies proximal to D10S1418.
(6) Haplotype g along with haplotype a does not cause the disease phenotype (Fig. 1). Therefore, haplotype g must be a non-NCRNA haplotype. Haplotype g must have resulted from a recombination

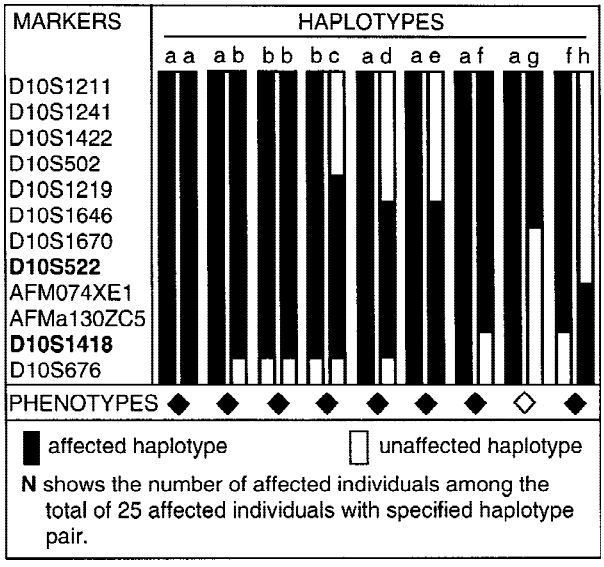

Fig. 1. Haplotypes observed in the NCRNA region in chromosome band 10q21 in the affected population. The historic founder haplotype is designated a, and different ancestral recombinant haplotypes are designated b–f. The new haplotypes created during parental gametogenesis in the present generation are designated g and h (see Fig. 2). Recombination events observed in haplotypes f and g define the current interval. Flanking markers are shown in bold.

event between markers D10S1646 and D10S1670, involving haplotypes f and n1, in individual I-1 (Fig. 2).

(7) Haplotype h along with haplotype f produces the disease phenotype (Fig. 1). Therefore, haplotype h must be a NCRNA haplotype. Haplotype h must have resulted from a recombination event between marker D10S522 and the NCRNA locus, involving haplotype a and n4, in individual I-4 (Fig. 2).

The NCRNA interval between the closest proximal and distal markers, D10S522 and D10S1418 is ~0.6–1.5 cM and contains markers AFM074XE1 and AFMa130ZC5. No recombination was observed with these two markers.

DISCUSSION

In an extensive study of 20 families with NCRNA, undertaken to refine the location of the NCRNA locus on 10q21, we used DNA samples from 106 individuals from an affected Iranian population. For this study, from a long founder disease haplotype, a set of 12 markers (haplotype a), covering ~9 cM, was chosen.

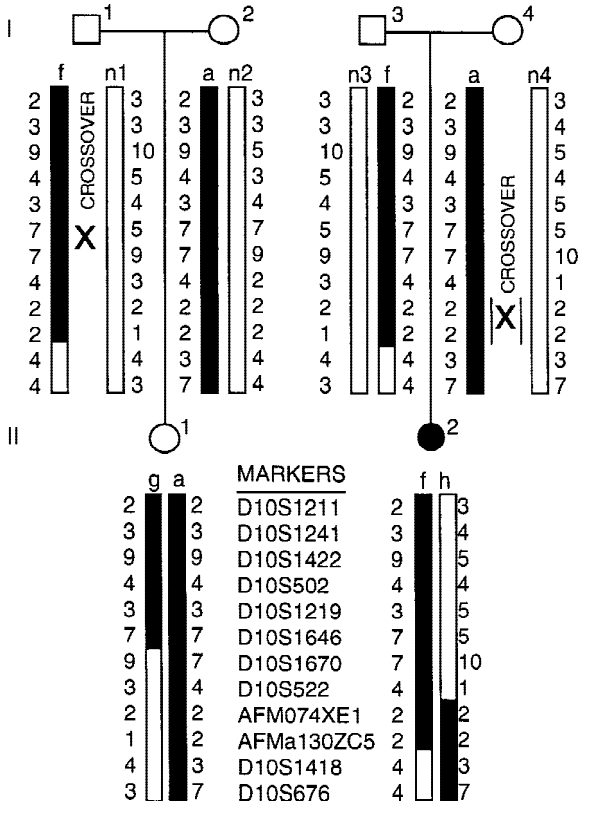

Fig. 2. Two new haplotypes were generated in the present generation. Haplotypes a and f are two ancestral NCRNA haplotypes; n1, n2, n3, and n4 are non-NCRNA haplotypes. A crossover between markers D10S1646 and D10S1670 involving haplotypes f and n1 in individual I-1 generated haplotype g. The normal phenotype in individual II-1 with haplotype pair ga indicates that g must have received the normal NCRNA allele from n1. Therefore, the NCRNA locus must be located distal to D10S1646. A crossover between marker D10S522 and the NCRNA locus involving haplotypes a and n4 in individual II-4 generated haplotype h. The disease phenotype in individual II-2 with haplotype pair fh indicates that she must be homozygous for the disease allele. Therefore, h must have received the disease allele from a, indicating that the NCRNA locus is distal to D10S522.

Across this founder haplotype we observed five historic and two new recombination events (Figs. 1, 2).

Haplotypes of the affected individual in Figure 2 are f and h, where f is the paternal disease haplotype and h is a new recombinant haplotype harboring a part of the maternal haplotype a, containing the disease gene. Haplotypes h and n4, share the same marker alleles. Haplotype n4 does not contain the NCRNA allele, yet individual II-2 must be homozygous for the NCRNA allele to have the NCRNA phenotype. Therefore, in addition to her paternal disease allele in f, she must have also received a disease allele from her mother. For this to occur individual II-2 must have received a new recombinant disease haplotype from her mother, i.e., an event that was generated by a crossover between the marker D10S522 and the disease locus during oogenesis (Fig. 2). Because individual I-4 is not informative for the distal markers, we cannot precisely determine the exact location of the crossover.

For the same reason, if it were not for the disease phenotype in II-2 in Figure 2, we would not have suspected the occurrence of a crossover distal to D10S522 in this family, on the basis of their haplotypes. Therefore, if individual II-2 had been evaluated prenatally using haplotype analysis, we would not have recognized her as affected and would have been surprised with a false negative diagnosis. To be sure that these results are not due to mistakes in phenotype ascertainment or mishandling of the blood or DNA samples, individuals I-3, I-4, and II-2 in Figure 2 were resampled to obtain DNA and their phenotypes reconfirmed.

Due to seven recombination events across this founder haplotype, the length of the disease haplotype has gradually decreased on either side in the study group (Fig. 1). However, the founder haplotype, or most of it, is observed in 100% of the NCRNA patients: Of 25 affected individuals studied, 7 were homozygous for haplotype a, 13 were heterozygous for a, 3 were heterozygous for b, and 1 was heterozygous for f (b and f have 11 and 10 of the 12 marker-alleles in a, respectively). The haplotypes of a number of the parents and/or normal sibs of the deceased NCRNA affected individuals also confirmed the association of a single founder haplotype with the NCRNA locus. This dramatic linkage disequilibrium provides evidence that a single founder mutation is most likely responsible for all of the cases among this Iranian population.

In this long haplotype only five ancestral recombination events have been detected, which have progressively reduced the founding haplotype on either side to a ~4.5 cM region defined by D10S1219 and D10S1418 (Fig. 1). However, one of the new recombination events has reduced the NCRNA region to a segment of ~1.5 cM flanked by D10S522 and D10S1418 (Figs. 1, 2). The extent of the linkage disequilibrium on NCRNA chromosomes and the comparison of historic and new recombinations in NCRNA suggests that the NCRNA mutation probably occurred, or drifted relatively recently, in a small number of founders of the study population. The high incidence of NCRNA in the affected population also suggests that the mutation likely was present among the small number of founders and before the population expansion a few centuries ago.

The incidence of NCRNA in the study population is ~1.1% with the calculated carrier frequency of ~20% [Ghiasvand et al., 1998]. The identification of the NCRNA haplotype, which was observed only on NCRNA chromosomes and not on non-NCRNA chromosomes, indicates that a relatively accurate genetic test can be developed for relatives with NCRNA and their spouses. Of course, there is always the possibility of recombination events occurring between the closest flanking markers and the disease gene, which could lead to misleading interpretations. Therefore, until the development of a direct diagnostic test for this disease, the use of an indirect linkage-based predictive test for this disease must be applied with caution. The information obtained during this study will form the basis for DNA-based predictive tests (carrier detection and prenatal diagnosis) for NCRNA in the affected population.

The identification of flanking markers for the autosomal recessive locus and the refinement of its localization to a 0.6–1.5 cM interval in the chromosome band 10q21, provides important initial data for physical mapping, cloning, and characterization of the gene. Cloning the NCRNA gene also may provide insight regarding the histopathology of retinal abnormalities and retinal development. Moreover, direct analysis of the NCRNA gene would show whether different forms of retinal nonattachment are allelic or are controlled by different loci. This would allow classification of the retinal nonattachment based on the primary genetic cause rather on subtle symptomatic or transmission differences.

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